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Effect of Silver Nanoparticles Synthesized by Pulsed Laser Ablation in Liquid on the Hematological, Hepatic, and Renal Functions of Albino Rats

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Abstract

Silver nanoparticles (Ag-NPs) have unique properties as antibacterial effects against locally isolated clinical *Escherichia coli*. In this study, the evaluated the antibacterial activity of AgNPs, which were synthesized by laser ablation, against locally isolated clinical *Escherichia coli* on nutrient agar media *in vitro*. Then assessed the toxicity of the bactericidal dose in albino rats *in vivo* with hematological, liver, and kidney functions as vital parameters. AgNPs were synthesized by pulsed laser ablation in liquid (PLAL). AgNPs' shape and nano size were characterized by atomic force microscopy (AFM), UV-vis spectroscopy, and scanning electron microscopy (SEM). UV peak absorption was observed at 434 nm, which produced an average particle diameter of 28.09 nm. The SEM and AFM topography images of the synthesized nanoparticles showed that they had a spherical shape. Also studied was the toxicity of the synthesized AgNPs *in vivo*. Four groups of albino rats were used. The liver and kidneys were found to be the main organs that accumulate AgNP. In this study, we examined the influence of intraperitoneally injected AgNPs on blood parameters (complete blood picture) and liver and kidney functions of albino rats. Blood ALP and serum test (GOT and GPT), serum urea and serum creatinine were analyzed at 15, 30, and 60 days after injection of 200 mg/kg of PLAL-synthesized AgNP. The results did not indicate statistically significant differences between the control and treatment groups in blood picture and enzyme levels tested that indicate proper liver function. Additionally, no differences in kidney functions were found during varying time intervals in the blood of rats. Thus, PLAL-synthesized AgNPs could inhibit Escherichia coli bacterial growth.

Keywords: Antibacterial, Silver nanoparticles, Toxicity, Liver function, Kidney function.

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تأثير جزيئات الفضة النانوية المُصنَّعة عن طريق الاستئصال بالليزر النبضي في السائل على وظائف الدم والكبد والكلى في الجرذان البيضاء

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الخلاصة

في هذه الدراسة ، قمنا بتقييم النشاط المضاد للبكتيريا لـ AgNPs ، التي تم تصنيعها عن طريق الاستئصال بالليزر ، ضد الإشريكية القولونية المعزولة محليًا على وسط أجار المغذيات في المختبر. قمنا بعد ذلك بتقييم سمية جرعة مبيد الجراثيم في الجرذان البيضاء في الجسم الحي مع وظائف الدم والكبد والكلى كمعايير حيوية. تم تصنيع AgNPs عن طريق الاجتثاث بالليزر النبضي في السائل (PLAL). يتميز شكل AgNPs وحجمه بالنانو من خلال الفحص المجهري للقوة الذرية (AFM) ، والتحليل الطيفي للأشعة فوق البنفسجية ، والمسح المجهري الإلكتروني (SEM). لوحظ امتصاص الذروة للأشعة فوق البنفسجية عند 434 نانومتر ، مما أدى إلى إنتاج متوسط قطر للجسيمات يبلغ 28.09 نانومتر. أظهرت صور التضاريس SEM و AFM للجسيمات النانوية المركبة أن لها شكلًا كرويًا. درسنا أيضًا سمية AgNPs المركب في الجسم الحي. تم استخدام أربع مجموعات من الجرذان البيضاء. تم العثور على الكبد والكلى ليكونا الأعضاء الرئيسية التي تتراكم AgNP. في هذه الدراسة ، قمنا بفحص تأثير AgNPs المحقونة داخل الصفاق على معايير الدم (صورة الدم الكاملة) ووظائف الكبد والكلى للفئران البيضاء. تم تحليل ALP في الدم واختبار المصل (GOT و GPT) ، ويوريا المصل والكرياتينين في الدم في 15 و 30 و 60 يومًا بعد حقن 200 مجم / كجم من AgNP المركب من PLAL. لم تشر النتائج إلى وجود فروق ذات دلالة إحصائية بين مجموعة التحكم ومجموعات العلاج في صورة الدم ومستويات الإنزيم التي تم اختبارها والتي تشير إلى وظائف الكبد السليمة. بالإضافة إلى ذلك ، لم يتم العثور على اختلافات في وظائف الكلى خلال فترات زمنية متفاوتة في دم الفئران. وبالتالي ، فإن AgNPs المركب بواسطة PLAL يمكن أن يمنع نمو الإشريكية القولونية.

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1. Introduction

Nanotechnology is the term that refers to the technology of synthesizing materials and devices in the nanometer range (from 1 to 100 nm) [1]. This new field of science has attracted considerable attention from the scientific community around the world because nanomaterials possess physical and chemical characteristics and functions that are completely different from the bulk material from which they are derived. The small size, large surface area, tailor ability, highly improved solubility, and multifunctionality of NPs open many new research avenues for biologists. The biological applications of this new technology are progressively expanding in different fields. Research dealing with these applications is concentrated mainly in the fields of application where biological science is facing difficulties, such as drug delivery, imaging for diagnoses, nanosensing for diagnoses, and antipathogenic agents for drug delivery [2-4]. One of the fields that involve tremendous work is the exploration of the antimicrobial activity potential of inorganic NPs. Different types of inorganic NP synthesized by different procedures have been investigated. NPs of gold, silver, titanium oxide, zinc oxide, iron oxide, and copper oxide were investigated [8–12]. For clinical applications, the toxicity and impact of these

nanomaterials on the environment remain major concerns. Many researchers have begun to analyze the toxicity of these and other nanomaterials to human cells, animal cells, plants, and aquatic environments [13–15]. The cost and time required to perform such assessments must also be examined [16]. On 7 April 2011, which is considered World Health Day, the World Health Organization (WHO) announced that resistance to antimicrobial agents represents one of the major problems facing the human health sector. In its report, the WHO described that "the emergence and spread of drug-resistant pathogens have accelerated. More and more essential medicines are failing. The therapeutic arsenal is shrinking. The speed with which these drugs are lost far outpaces the development of replacement drugs. In fact, the R&D pipeline for new antimicrobials has practically run dry." Accordingly, this issue needs to be addressed carefully by exploring nanotechnology and what nanotechnology offers in this field. In particular, silver nanoparticles (AgNPs) show considerable antibacterial activity against Gram-positive and Gram-negative bacteria, making them a good candidate to overcome the issue of drug resistance [17]. The toxicity problem persists in research on the effect of nano-silver on bacteria, cells, and higher organisms and needs further understanding; however, this situation can be explained and answered with more carefully designed studies. The therapeutic window for silver is narrower than often assumed, and the risks to humans and the environment are probably limited [18].

Silver ions and their compounds are highly toxic to microorganisms, exhibiting strong biocidal effects on many species of bacteria but low toxicity toward animal cells. Therefore, silver ions, an antibacterial component, are employed in formulations of dental resin composites, bone cement, ion exchange fibers, and coatings for medical devices.

AgNPs exhibit new optical properties that are not observed in molecules and bulk metals. One example is the presence of an absorption band in the visible-light region. This band appears due to the surface plasmon oscillation modes of conduction electrons, coupled through the surface to external electromagnetic fields [19]. The surface plasmon resonance and the large effective scattering cross section of individual AgNPs make them ideal candidates for molecular labeling, where phenomena such as surface-enhanced Raman scattering can be exploited [20].

In this work, we focused on prepared AgNPs, one of the most important NPs commonly used in various fields, such as cosmetics, biomedicine, and antibacterial agents. Many products contain AgNPs, such as toothbrushes, so they may transfer into the bloodstream to body tissues through different routes. The accumulation of AgNPs in the body may lead to toxicity. Also, the AgNPs was prepared by the PLDIL method and determined their toxic effects on human tissues, such as the blood, kidney, and liver

2. Materials and Methods

2.1 Synthesis and characterization of AgNPs

Pulsed laser ablation in liquid (PLAL) was performed to synthesize AgNPs [21, 4]. In summary, a pure and polished silver metal plate was placed in a glass beaker containing 10 mL of double-distilled deionized water. The ablation source was a 1064 nm Nd-YAG laser (type HUAFEI). The laser pulse duration and repetition rates were 10 ns and 10 Hz, respectively. The laser energy was 600 mJ/pulse. The number of pulses utilized was 1000 pulses (Figure 1). The preparation of the sample for the examination of atomic force microscopy (AFM) was carried out according to Angstrom Advances Inc., USA, [22]. AA2000 AFM was used to indicate NP formation. The NP size distribution was examined with CSPM software (Angstrom Advances Inc., USA). The maximum absorbance of the NP solution was determined by using a UV-visible

double-beam spectrophotometer (CECIL, C. 7200, UK). The silver concentration in the prepared AgNP solution was estimated by atomic absorption spectroscopy (AAS model GBS 933, Australia).

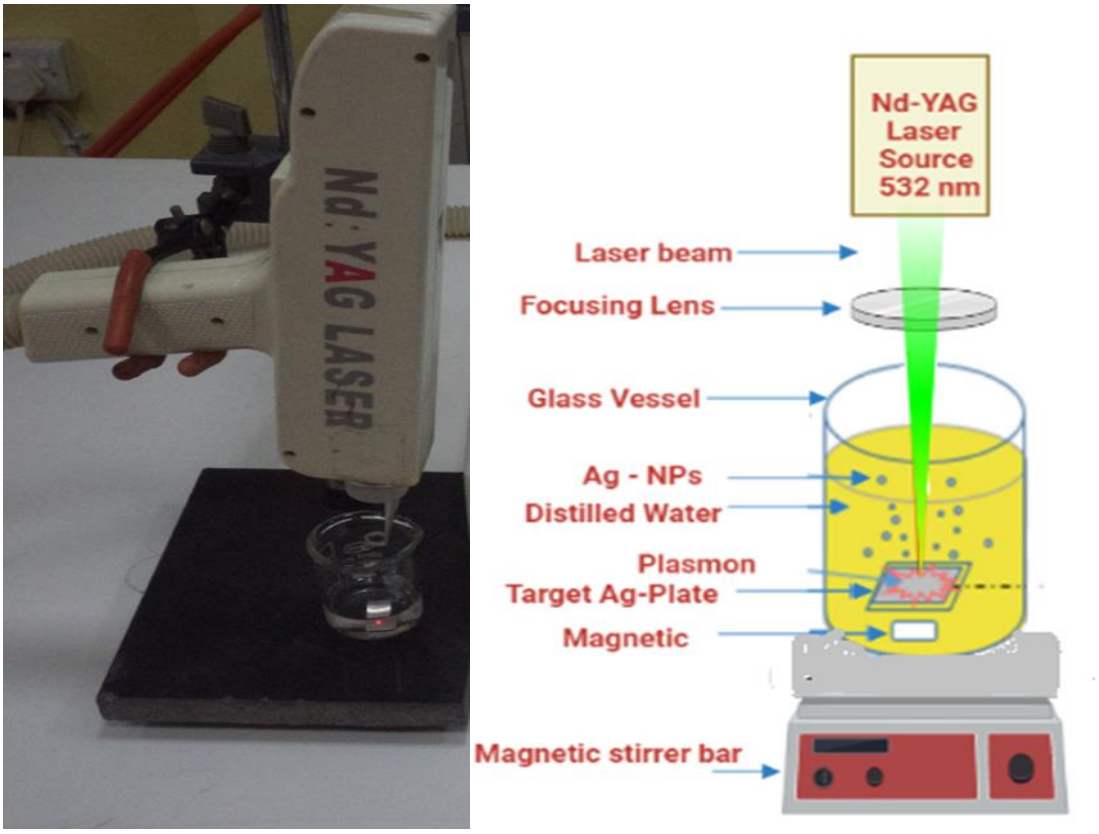

Figure 1: (a) Photographic image of the PLAL technique. (b) Schematic of the experimental setup for AgNPs prepared by PLAL [4].

2.2 Characterization of AgNPs

AgNPs were characterized for their optical properties by a UV-Vis spectrophotometer (UV-VIS SP8001, Metertech), Lambda-19, HACHLANGE DR-5000 type spectrophotometer. AFM (Digital Instruments Nanoscope II) and a scanning probe microscope (AA2000) were used for topography property measurements. Scanning electron microscopy (SEM, VEGA Easy Probe) was used to examine the morphological changes of the bacterial cells before and after treatment with AgNPs.

2.3 Evaluation of the antibacterial activity of AgNPs

The antibacterial activity of AgNPs was determined by the agar-well diffusion method. The cell culture suspension was adjusted by comparing it to the 0.4–0.5 McFarland scale standard. About 100 ml of the bacterial suspension was spread in MHA using a spreader and left for 10 min to settle. Approximately 50 µL of silver nitrate and AgNP solution was poured into each well and incubated for 24 h at 37 ° C. The diameter of the inhibition zone was calculated and recorded as the mean ± SE of the triplicate experiment. All samples were tested in triplicate [23,24].

2.4 SEM

SEM was performed to examine the morphological changes of bacterial cells before and after treatment with AgNP [25].

2.5 In vivo experiment

Eight-week-old adult male Wistar rats (150–200 g) were obtained from the Animal House Facility of the Veterinary College/University of Baghdad and housed at the same facility in a well-ventilated room at 25 ℃ ± 2 ℃ under a 12 h light/dark cycle in standard polypropylene cages. The in vivo experiment was carried out according to the animal care code implemented by the ethical community of animal care of the Iraqi Center for Cancer and Medical Genetic Research. All in vivo experiments were carried out in the animal house unit of the Iraqi Center for Medical Genetic Research and Cancer (the approval code was ACQ-22/2019 granted on February 2, 2019). The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water. The experimental design was carried out as follows: 14 male Wistar rats were divided into two groups. Group I consisted of 16 animals that served as the control treated with distilled water. Group II consisted of 20 animals and served as the AgNP challenged group. Animals in group II were administered AgNP intraperitoneally at a concentration equal to 200 µg/kg body weight. The dose was administered once daily for 60 consecutive days. After the end of the dosing period, animals from groups I and II (four and five animals, respectively) were randomly selected and sacrificed. This was carried out after 0 (an hour), 15, 30, and 60 days of AgNP administration. Animal blood was collected by direct heart puncture, and animal organs were preserved in buffered formalin for histopathological diagnosis [26].

2.6 Hematological parameters

Red blood cell packed volume (PCV) was determined according to the standard procedure [27]. The white blood cell count (WBC) and the differential count of WBC were measured according to Blumenreich (1990) [28].

2.7 Blood and serum tests

The following assays were performed on the blood and serum of the control and treated animals: blood urea test, serum creatinine test, serum glutamic oxalacetic transaminase (GOT) activity, serum glutamate pyruvate transaminase (GPT) activity, and serum alkaline phosphatase (ALP) activity. All these tests were carried out using Cypress Diagnostic kits (CYPRESS DIAGNOSTICS, Belgium) according to the manufacturer's recommended protocol.

2.8 Statistical analysis

One-way analysis of variance (ANOVA) was performed and significant differences were calculated at probability levels of 0.05 and 0.01.

3. Results and Discussion

3.1 Characterization of AgNPs

PLAL was carried out to synthesize AgNPs. The ablation setup and laser parameters used were suitable for producing the NPs. Colorless double-distilled water, which was the reaction environment, turned to a gray, yellowish solution at the end of the ablation process. The intensity of the color of the solution was dependent on the ablation time (Figure 2). This color change in the solution indicated the formation of AgNPs. This formation was easily followed and observed by UV-vis spectroscopy, which indicated a peak absorbance at 435 nm (Figure 3). The size distribution of the synthesized NPs has obtained from the C scanning probe microscope (CSPM) software (Figure 4). The particle size distribution (blue columns), the analysis of the accumulation distribution curve (red line) of the granularity and the AFM topography image indicated that this process produced SNPs with an average diameter of 28.09 nm. The AFM topography image of the synthesized nanoparticles showed a spherical to

amorphous shape, which indicated particle aggregation (Figure 5). PLAL represents one of the methods used to synthesize metal NPs such as AgNPs. Compared to the other methods used for AgNP formation, PLAL is less costly, less time-consuming, and produces less contaminated products because no chemical precursors are required; the final product solution is a homogeneous colloidal of NPs [29]. The characteristics of the NPs produced using PLAL depend on the conditions of the laser used. Nanosecond, femtosecond, and picosecond lasers were utilized in the formation of colloidal AgNPs [30]. In this study, the nanosecond pulsed duration was used to produce AgNPs with nano size [31].

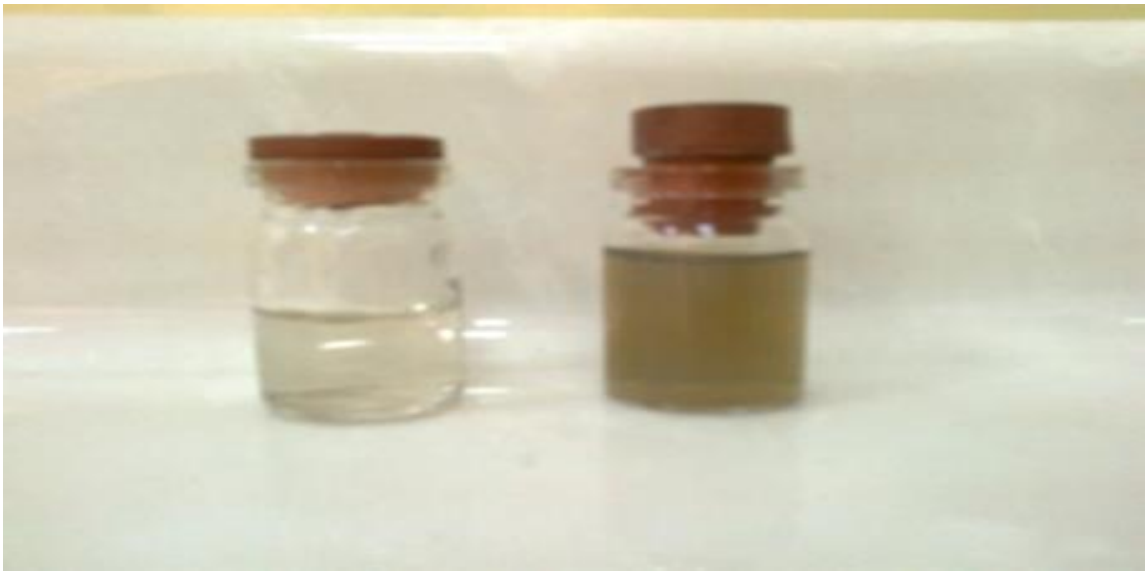

Figure 2: Gap-nano-electronic NP colloidal comparing the color of two solutions produced by different laser ablation times.

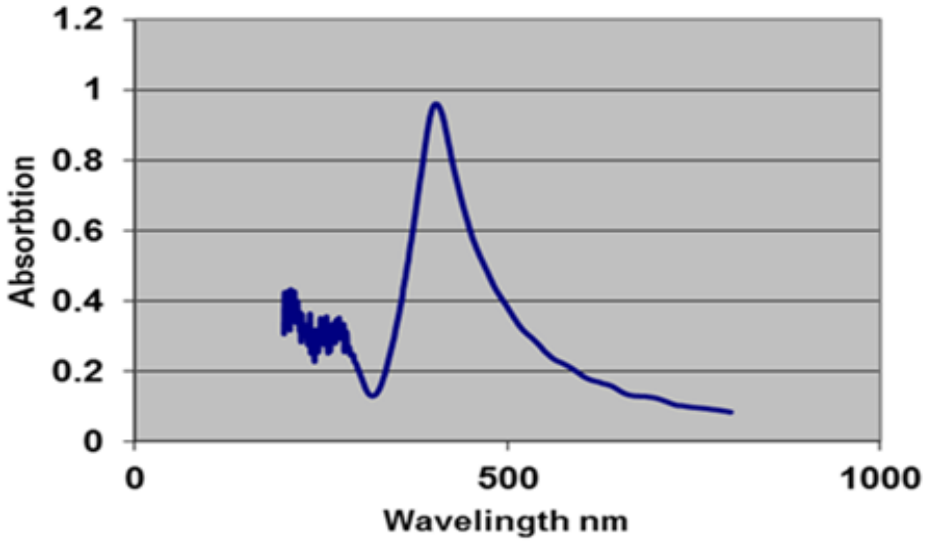

Figure 3: UV-vis scanner spectrophotometer absorption indicates a maximum absorbance at 420 nm.

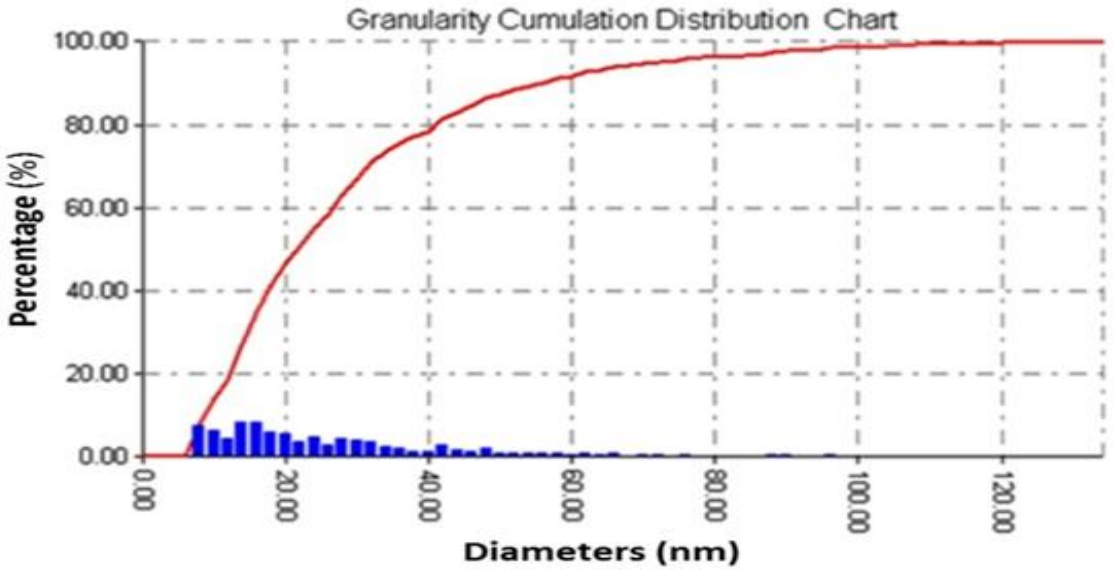

Figure 4: Scanning probe microscope (CSPM) software analysis of particle size distribution (average diameter = 28.09, 10% diameter = 8.00 nm, 50% diameter = 20.00 nm, and 90% diameter = 54.00 nm). The blue columns represent the diameter of the particles, and the red curve represents the percentage of accumulation of garrule.

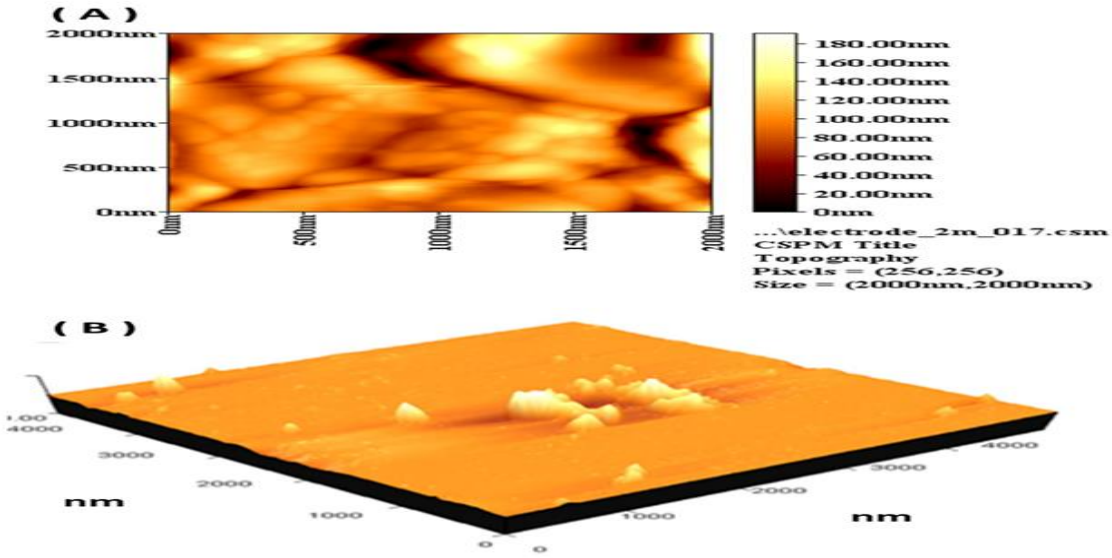

Figure 5: Atomic force microscope imaging for silver nanoparticles synthesized by pulsed laser ablation in liquid. A: Topography image with particle size scale bar. B: 3D image of the synthesized nanoparticles.

Figure 6 shows obvious changes in the shape of enteric bacterial cells after treatment with AgNP. The distortions were due to exposure of AgNP to oxygen and the release of Ag + ions during the preparation of the bacterial viability assay and fluorescence microscopy; these ions later interacted with negatively charged bacterial surfaces [25].

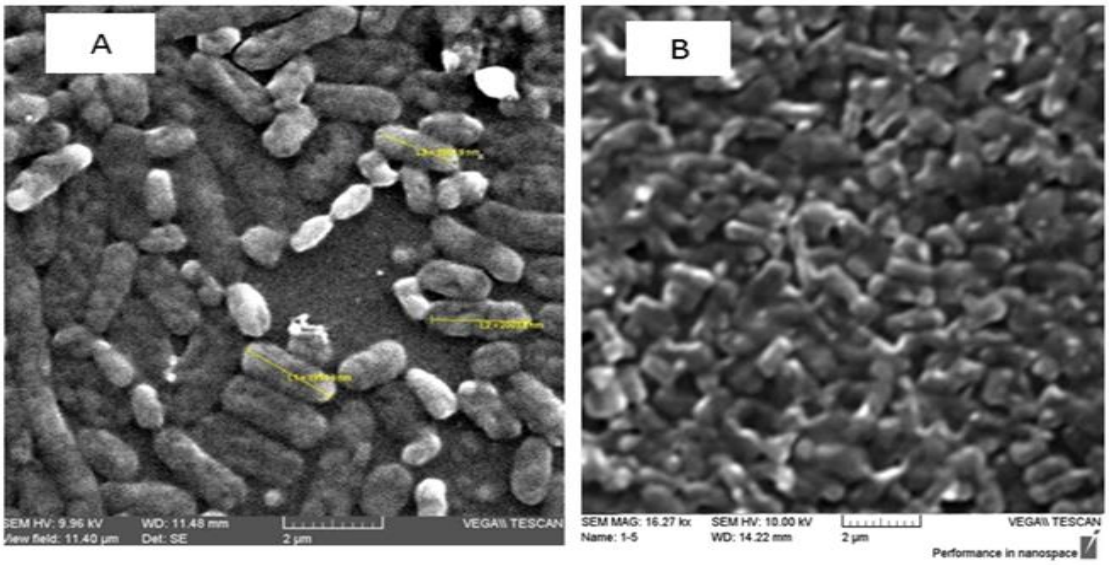

Figure 6 : SEM images of *E. coli* bacteria, A: untreated cells; B: AgNP-treated cells. Locally isolated E. coli bacterium treated with AgNP synthesized by PLAL.

3.2 Blood, Serum and Hematological test

Figures 7 and 8 show that AgNPs had no toxic effect on the activity of enzymes (GOT and GPT), thus representing the activity of the liver and kidneys. The activity of the enzymes increased, which was consistent with some studies [32, 33]. The ability of AgNPs to modulate enzyme activity is attributed to their affinity for thiol groups [34–36]. The thiol groups in the enzymes made them attractive to AgNPs, resulting in the formation of complexes and consequent modulation of enzyme activity [37].

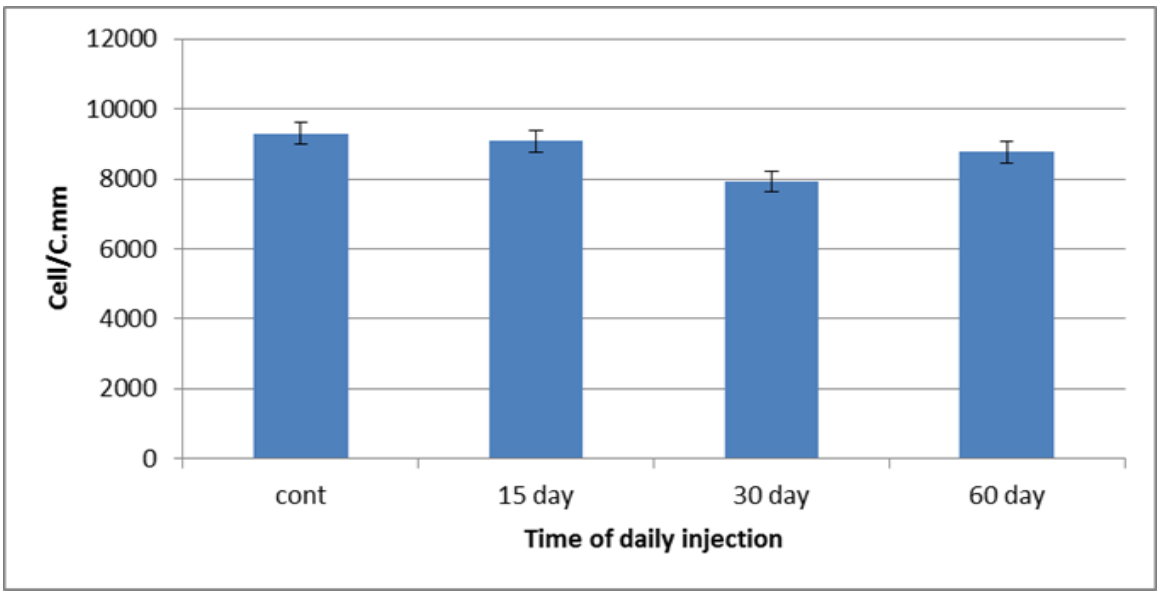

Figure 7 : White blood cell counts in rats injected intraperitoneally with 200 µg/kg BW/day AgNPs daily after 15, 30, and 60 days. Control animals were injected with 200 µL of distilled water.

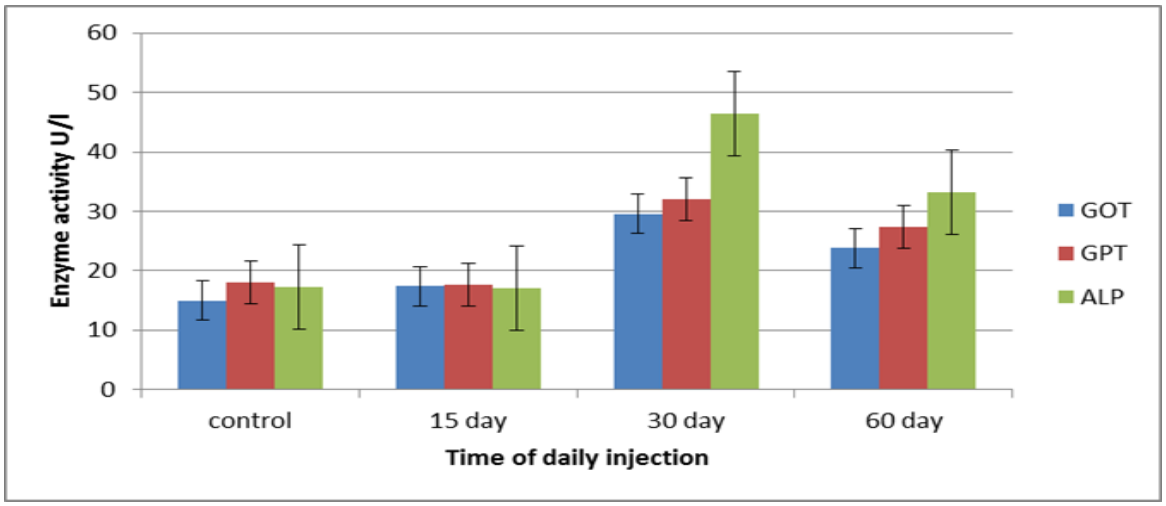

Figure 8 : Levels of glutamic oxalacetic transanunase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (APL) enzymes in the serum of rats after daily intraperitoneal injection of 200 µg/kg BW/day AgNPs after 15, 30, and 60 days. Control animals were injected with 200 µL of distilled water.

AgNP did not show any modulation or toxic effect on some liver and kidney functions (Figures 7, 8, and 9), consistent with the literature [38]. This study found that HP caused hemolytic anemia through hematologic toxicity. They linked this to the production of free radicals that damaged cell membranes and caused bone marrow necrosis, which decreased the number of hematopoietic cells [27, 39]. However, HP-induced photodermatitis in rats resulted in a significant increase in total WBC count. It could be because HP has an immunomodulatory effect on lymphocytes, which leads to more white blood cells and a more robust immune response [28, 29]. Furthermore, HP-produced bone marrow necrosis plays an essential role in the development of leukocyte granule marrow disease [30]. Notably, the application of AgNP coadministered with HP or alone in rats resulted in a considerable improvement in all hematologic parameters, nearly to the same level as in healthy control rats.

This outcome was consistent with the positive effects of AgNP on hematobiochemical recovery in disease [31] as well as the critical role of AgNPs in the promotion of hematopoiesis. Therefore, AgNPs play an essential role in controlling the hematopoietic process, possibly by correcting abnormalities.

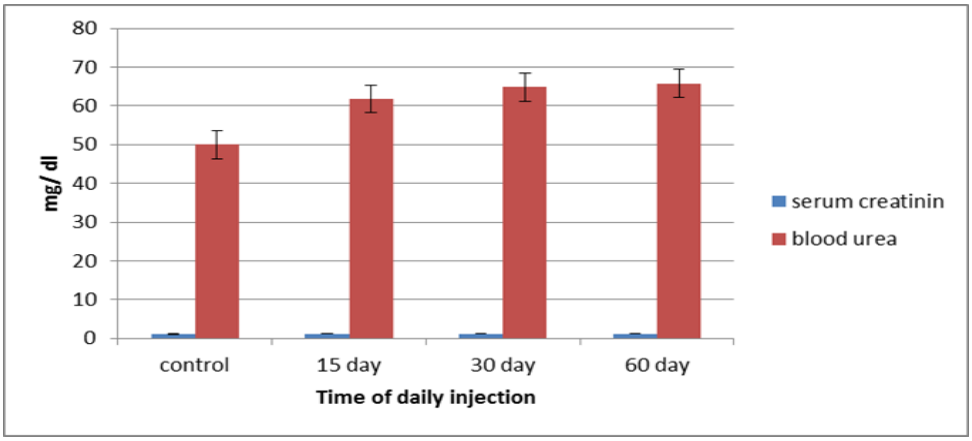

Figure 9: Serum creatinine and serum urea levels of rats after daily intraperitoneal injection of 200 µg/kg BW/day AgNPs after 15, 30, and 60 days. Control animals were injected with 200 µL of distilled water.

Figures 10 and 11 show the ability of AgNP to increase the percentage of some hematological parameters, such as neutrophil and lymphocyte counts, while some differential counts of WBC, such as monocyte, basophil, and eosinophil ratios, were not affected by AgNP.

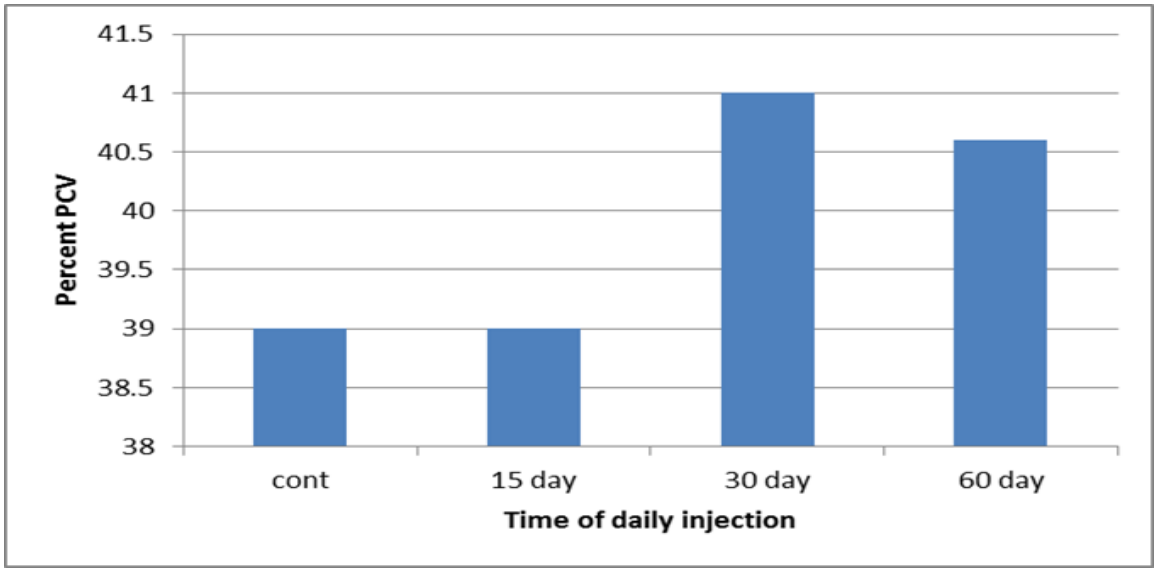

Figure 10 : Cell volume of rats' blood packed after daily intraperitoneal injection of 200 µg/kg BW/day AgNPs after 15, 30, and 60 days. Control animals were injected with 200 µL of distilled water.

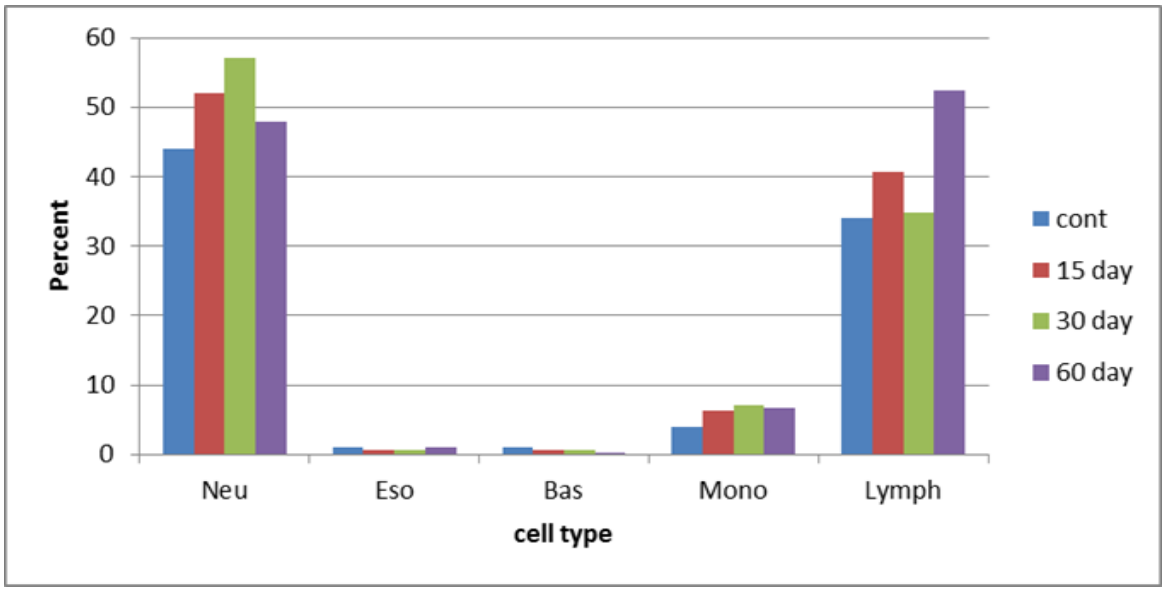

Figure 11: Differential count of leukocytes in rats' blood after daily intraperitoneal injection of 200 µg/kg BW/day AgNPs after 15, 30, and 60 days. Control animals were injected with 200 µL of distilled water.

3.3 In vivo experiment

In vivo tests were conducted to assess the toxicity of using an NP mouse model on the basis of the in vitro findings. The result did not show harmful effects and there were no statistically significant variations in body weight between AgNP-treated animals and control mice (P 0.05). After the experiment, a necropsy revealed no macroscopic organ alterations in the treated groups. Furthermore, to assess possible toxicity, histological pictures of the examined organs were obtained. Figures 12, 13, and 14 show that AgNP-treated mice did not show visible

damage or histological abnormalities. Pathology findings revealed poisoning at the macroscopic and ocular levels. In the organs studied, no significant pathological alterations or organ damage were discovered. As a result, the animals' organs (liver, kidneys, lung, spleen, and brain) did not show any histopathological alterations after being exposed to AgNPs, indicating that the use of AgNPs for in vivo applications might be beneficial.

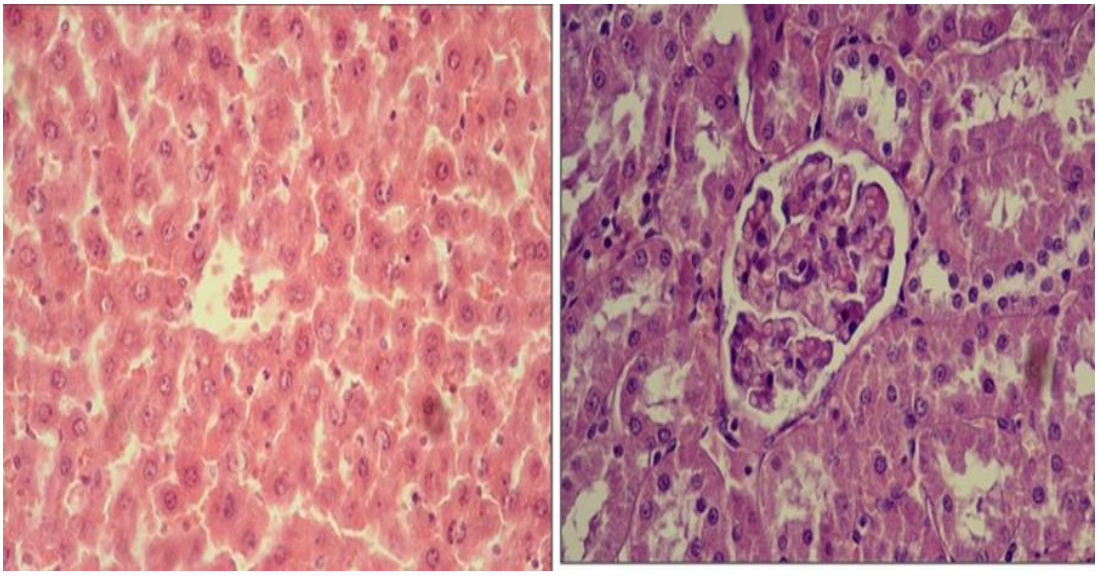

Figure 12 : Histological section in liver (left) and kidney (right) of male rat intraperitoneally injected with 200 µg/kg BW/ day AgNP synthesized by pulsed laser ablation for 2 months, group IV, showing no clear pathological changes in liver and kidney parenchyma (H & E stain X 400).

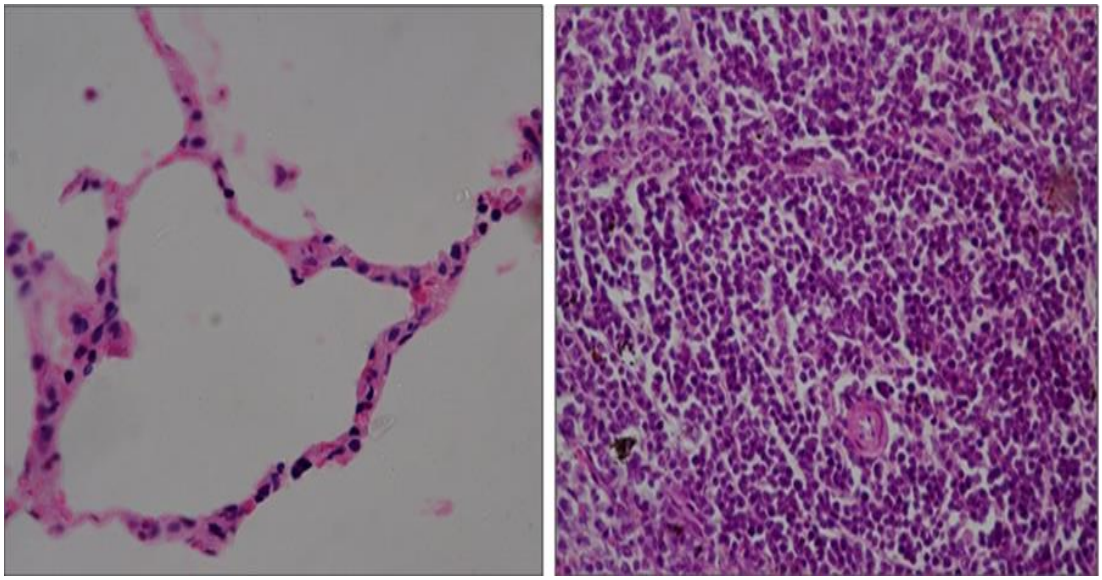

Figure 13 : Histological section in the lung (left) and spleen (right) of male rats intraperitoneally injected with 200 µg/kg BW/ day AgNP synthesized by pulsed laser ablation for 2 months, group IV, showing no clear pathological changes in lung tissue and normal lymphoid follicle of spleen and white pulp (H & E stain X 400).

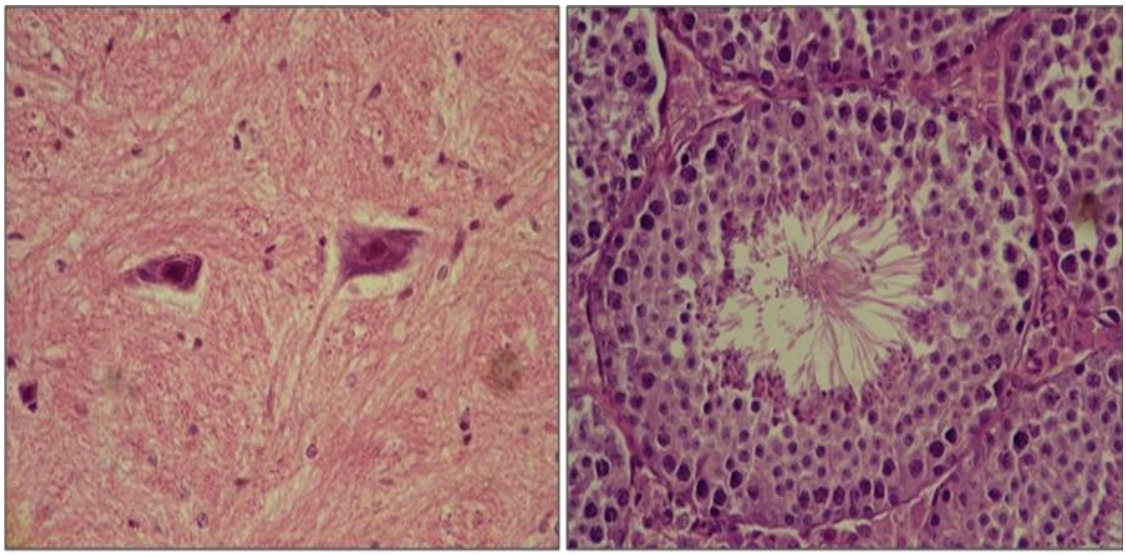

Figure 14: Histological section in the brain (left) and testes (right) of male rat intraperitoneally injected with 200 µg/kg BW/day AgNPs synthesized by pulsed laser ablation for 2 months, group IV, showing no clear pathological changes in brain tissue and normal cells of the seminiferous tubules and interstitial cells (Leydig) (H & E stain X 400).

3.4 Antibacterial activity of AgNPs

The results in Table 1 showed the effects of silver nitrate and AgNO3 by diffusion of agar wells at different concentrations of 5 mM (12.5%, 25%, 50%, and 100%) against two pathogenic bacteria. The highest inhibition zone was 19.66 mm at 100% cons. (Stock), and the lowest inhibition zone was 8 mm at 20% to silver nitrate. The NPs showed a high inhibitory effect compared to that ~~of~~ silver nitrate. The highest effect was the synthesized AgNP, which revealed that the average diameter of the inhibition zone reached 22.33 mm. This result may be attributed to the smallest-sized spherical particles of AgNPs because of the high surface-to-volume ratio. The small NPs released more silver cations than the large NPs, and the former proved to be more effective in killing bacteria than the large particles [40]. Several studies suggested the high impacts of the use of NPs against *E. coli*, including the induction of damage to the cell wall of the bacteria, increasing membrane permeability, and causing cell death [41, 42].

Table 1: Antibacterial activity of silver nitrate and silver nanoparticles against *E. coli* bacterium

Bacterial Isolation	Inhibition zone of silver nitrate and AgNP concentration in millimeter				
	20%	40%	60%	80%	100%
E.coli	Silver nitrate				
	8.00	8.33	9.00	14.33	19.66
	Silver nanoparticles (AgNPs)				
	8.33	11.66	12.66	17.33	22.33

4. Conclusions

The study demonstrated that AgNP plays an important role as an antioxidant and anti-inflammatory in the mice investigated. Furthermore, after HP-induced photosensitivity in rats, AgNP played an important role in improving hemopoiesis and hemoprostat function. AgNP did not show any histopathological alterations in tested animals. Thus, AgNP can be used as a biological enhancement agent for many aspects of the body function of photosensitized rats.

Conflicts of interest:

The authors declare that they have no conflict of interest.

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